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PAPER

Dairy augmentation of total and central fat loss in obese subjects

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BACKGROUND AND OBJECTIVE: We have previously demonstrated an antiobesity effect of dietary Ca; this is largely mediated by Ca suppression of calcitriol levels, resulting in reduced adipocyte intracellular Ca^{2+} and, consequently, a coordinated increase in lipid utilization and decrease in lipogenesis. Notably, dairy Ca is markedly more effective than other Ca sources.
DESIGN: Obese subjects were placed on balanced deficit (−500 kcal/day) diets and randomized to control (400–500 mg Ca/day; $n=16$) or yogurt (1100 mg Ca/day; $n=18$) treatments for 12 weeks. Dietary macronutrients and fiber were held constant at the US average.
MEASUREMENTS: Body weight, body fat and fat distribution (by dual-energy X-ray absorptiometry), blood pressure and circulating lipids were measured at baseline and after 12 weeks of intervention.
RESULTS: Fat loss was markedly increased on the yogurt diet (-4.43 ± 0.47 *vs* -2.75 ± 0.73 kg in yogurt and control groups; $P<0.005$) while lean tissue loss was reduced by 31% on the yogurt diet. Trunk fat loss was augmented by 81% on the yogurt *vs* control diet ($P<0.001$), and this was reflected in a markedly greater reduction in waist circumference (-3.99 ± 0.48 *vs* -0.58 ± 1.04 cm, $P<0.001$). Further, the fraction of fat lost from the trunk was higher on the yogurt diet *vs* control ($P<0.005$).
CONCLUSION: Isocaloric substitution of yogurt for other foods significantly augments fat loss and reduces central adiposity during energy restriction.
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Keywords: calcium; dairy; energy restriction; fat loss; yogurt

Introduction

A substantial body of evidence has emerged over the last 3 y in support of an 'antiobesity' effect of dietary calcium and dairy products.[1–7] Increasing dietary calcium in the absence of energy restriction appears to result in a repartitioning of dietary energy from adipose tissue to lean body mass, resulting in a net reduction in fat mass in both mice and humans.[1,8] Furthermore, increasing dietary calcium intake during energy restriction resulted in a marked augmentation of body weight loss and fat loss in both mice and humans.[3,5,6,9–11] Notably, when dairy is the source of the calcium, these effects are more pronounced, suggesting that additional components of dairy contribute to this effect. These findings are supported by epidemiological observations from NHANES III,[1] The Quebec Family Study,[12] The Continuing Survey of Food Intake by Individuals (CSFII),[13] the CARDIA study[14] and the HERITAGE Family Study.[15]

We recently reported that increasing dietary calcium significantly augmented weight and fat loss secondary to a fixed level of energy restriction (a deficit of 500 kcal/day).[11] Increasing dietary calcium intake from ~400 to ~1200 mg/day during energy restriction resulted in 26 and 38% increases in weight and fat loss, respectively, while utilizing dairy foods rather than calcium supplements without changing energy or macronutrient intake resulted in 70 and 64% increases in weight and fat loss. Moreover, fat loss from the trunk region was markedly increased, from 19% of total fat loss on a low-calcium diet to 50 and 66% on high-calcium and high-dairy diets, respectively.[11]

Our previous studies of the role of intracellular Ca^{2+} signaling and calcitrophic hormones in regulating human adipocyte metabolism provide a logical framework for understanding this antiobesity effect of calcium and dairy products. These mechanisms have recently been reviewed.[3,4] In brief, the increase in calcitriol (1,25-dihydroxyvitamin D), which occurs in response to low-calcium diets, acts on adipocytes via a specific membrane vitamin D receptor to

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increase Ca^{2+} influx. The resultant increase in intracellular Ca^{2+} exerts a coordinated effect on adipocyte lipid metabolism, serving to stimulate lipogenic gene expression and lipogenesis and to suppress lipolysis, thereby increasing lipid filling and adiposity. In addition, calcitriol also acts via a nuclear vitamin D receptor to suppress the expression of adipocyte uncoupling protein 2 (UCP2) expression, thereby limiting mitochondrial fatty acid transport and oxidation.[16] In contrast, high-calcium diets suppress calcitriol levels, resulting in reduced lipogenic gene expression and increases in lipolysis and UCP2 expression, thereby reducing lipid filling and adiposity. As noted above, dairy products exert significantly greater effects than calcium *per se*, and this augmented effect is likely due to additional bioactive compounds, such as angiotensin converting enzyme inhibitors, which are found in dairy, as well as to the rich concentrations of branched-chain amino acids, which may act in concert with calcium to attenuate adiposity.[3]

Our previous prospective clinical trial utilized a mixture of milk, cheese and yogurt as dairy sources and, accordingly, it was not possible to attribute an antiobesity effect to any single dairy product. Accordingly, the present study was conducted to determine if the utilization of a single dairy product (yogurt) in a highly structured diet would result in a comparable augmentation of weight and fat loss to that observed with a mixture of dairy products.

Materials and methods

Study design

This study was designed to determine whether a fermented dalry product (yogurt) will accelerate weight and fat loss induced by caloric restriction in 38 otherwise healthy obese adults. Subjects were studied for a 2-week lead-in period to establlsh their current calorlc requirements and provide an opportunlty for baseline dietary and physiological assessment, and then randomized to the following outpatient dietary regimens for 12 weeks: (1) a control diet providing a 500 kcal/day deficit, 0–1 servings of dairy products/day and 400–500 mg calcium per day; or (2) a yogurt diet providing a 500 kcal/day deficit and containing three daily six-ounce servings of a commercial fat-free yogurt (Yoplait Light), to bring the total calcium intake from 500 to 1100 mg/day. All calcium in both diets was derived from food sources. The control diet incorporated as a placebo three daily servings of a sugar-free, calcium-free, prepackaged flavored gelatin dessert containing 10 kcal/serving.

Subjects were provided individual instruction, counseling and assessment from the study dietitian regarding dietary adherence and the development and reinforcement of strategies for continued success; although the diets were individualized to achieve a 500 kcal/person/day deficit, comparable advice was given to patients in both treatment groups and diets were monitored weekly. All subjects maintained complete diet diaries, and compliance was assessed by weekly subject interview and review of the diet diary and product return (analogous to pill-counts). Physical activity and tobacco use was assessed using standard questionnaires and maintained at prestudy (baseline) levels throughout the study.

Body weight and waist circumference were measured weekly, with subjects wearing street clothes with no shoes, outerwear or accessories. Body fat was measured at the beginning of the study and at week 12 using dual-energy X-ray absorptiometry (DEXA); DEXA was also utilized to ascertain regional fat loss (abdominal fat *vs* other regions). Blood pressure and fasting levels of circulating insulin, glucose, glucose:insulin ratio (as a screening index of insulin sensitivity) and lipids (triglycerides, and total and HDL cholesterol) were measured at the same time points (baseline and week 12).

Subjects

A total of 38 otherwise healthy, obese adults ranging in age from 18 to 50 y were initially enrolled and 34 completed the study; there were no significant differences between completers and noncompleters for any parameter studied. All noncompleters left the study due to scheduling conflicts ($n = 3$) or relocating out of state ($n = 1$). Of the four subjects who did not complete the study, three were assigned to the control group and one to the yogurt group, leaving sample sizes of 16 and 18 in the control and yogurt groups, respectively. All subjects had an lnltial body mass index (BMl) of 30.0–39.9 kg/m^2, a low-calclum dlet (500–600 mg/day, as determined by food frequency and diet history) at study entry, no more than 3 kg weight change over the preceding 12 weeks and no recent (12 week) changes in exercise frequency or intensity. Patients were excluded from participation if they required the use of oral antidiabetic agents or insulin; utilized obesity pharmacotherapeutic agents and/or herbal preparations intended for the management of obeslty; utilized calcium supplements; reported adverse responses to dairy products; had a history of significant endocrine, hepatic or renal disease; were pregnant or lactating; or suffered any form of malabsorption syndrome. Baseline subject characteristics are summarized in Table 1. This research was approved from an ethical standpoint by the Institutional Review Board of the University of Tennessee; informed consent was obtained from all subjects and the research was conducted in accordance with the ethical standards outlined in the Helsinki Declaration.

Diets

Baseline dietary assessments (diet records) were conducted by the project dietitian during the 2-week lead-in period to provide an initial estimate of a maintenance level of caloric intake. This was refined by calculating energy needs using World Health Organization equations for calculation of basal

Table 1 Baseline patient characteristics

	Control	*Yogurt*
Gender	14F, 2M	13F, 5M
Age (y)	42±6	39±10
BMI (kg/m^2)	33.2±0.9	32.1±0.4
Systolic blood pressure (mmHg)	123±11	124±11
Diastolic blood pressure (mmHg)	82±8	82±8
LDL cholesterol (mg/dl)	159±25	158±21
HDL cholesterol (mg/dl)	45±12	46±11
Triglycerides (mg/dl)	136±25	121±22

Table 2 Diet characteristics

	Control	*Yogurt*
Calcium intake (mg/day)	495±28	1077±22
Energy intake (kcal/day)	1303±190	1437±190
% Energy from fat	30±3	30±4
% Energy from protein	18±3	18±2
% Energy from carbohydrate	52±6	52±5

metabolic rate (BMR), which were then adjusted for activity level to provide an estimate of total daily energy expenditure (TDEE). TDEE was estimated as 1.3 × BMR for obese patients engaged in mild daily activity and 1.5 × BMR for those engaged in strenuous daily activity. Discrepancies between estimated TDEE and baseline caloric intake were resolved, if necessary, by repeat diet records reviewed by the project dietitian. Based on this initial estimate of caloric needs, a food exchange-based diet was prescribed to produce a caloric deficit of ~500 kcal/day. The diets for the treatment arms were constructed to provide comparable levels of macronutrient and fiber, to approximate the average consumption in the US (fat ~35% of total kilocalories, carbohydrates ~49%, ~protein 16%, fiber 8–12 g/day). Nutritional supplements were not permitted, and caffeine intake was maintained at a constant level (individualized for each patient, based on baseline assessment). Diets were prescribed and monitored as noted above, and the key characteristics of diets achieved by subjects are shown in Table 2.

Assessment

Body weight was measured with a calibrated scale and height measured with a wall-mounted stadiometer with subjects in street clothes with no outerwear or shoes. BMI was calculated via standard equation (kg/m^2). Waist circumference was measured in the standing position, with measurements obtained midway between the lateral lower rib margin and the iliac crest. The measurements were taken mid-exhalation, and the average of two readings was recorded.

Total fat mass and percent lean and fat mass were assessed via DEXA (Lunar Prodigy) at baseline and at week 12 of study. The instrument was calibrated and a phantom was assessed daily to determine if any drift had occurred; spine phantom variation was less than 1.8% throughout the study.

Blood pressure and heart rate measurements were taken with the subject seated in an upright position in a chair for at least 5 min with the arm supported at heart level. Blood pressure was measured with an appropriately sized cuff using a standard, calibrated sphygmomanometer on the same arm for every measurement. Two readings, at least 1 min apart, were taken and the average value reported.

Plasma glucose was determined using a glucose oxidase method and insulin via standard radioimmunoassay using commercially available kits (Linco Research Inc., St Charles, MO, USA). Fasting lipid profiles (cholesterol, LDL cholesterol, HDL cholesterol and triglyceride) were assessed using standard clinical techniques. Circulating glycerol[17] was assessed at weeks 0 and 12 as an index of whole-body lipolysis.

Statistics

Data were assessed via multivariate MANOVA using SAS-PC software to facilitate evaluation of both the repeated-measures and independent group comparisons inherent to this study design. Only subjects who completed the entire study ($n = 34$) were included in the data analysis. All data are presented as mean±s.e.m.

Results

As expected from the experimental design, all participants lost body weight and body fat due to the daily energy deficit of 500 kcal/day. However, both weight and fat (via DEXA) loss were significantly increased by the yogurt diet compared to the control diet (Figure 1). Participants on the low-calcium control diet lost 4.99±0.5 kg of body weight, while this was increased by 22% for those on the yogurt diet to 6.63±0.6 kg ($P<0.01$, Figure 1, top panel). Fat loss followed a similar trend, although there was a greater augmentation of fat loss compared to weight loss. Participants on the control diet lost 2.75±0.73 kg fat, while those on the yogurt diet lost 4.43±0.47 kg ($P<0.005$, Figure 1, middle panel). The disparity between the extent of augmentation of weight loss (22%) *vs* fat loss (61%) by the yogurt diet may be attributable to the significant reduction in the loss of lean body mass during energy restriction in participants on the yogurt diet (−1351±156 g) *vs* the control diet (−1968±212 g) ($P<0.05$, Figure 1, bottom panel).

Consistent with our previous observations, trunk fat loss was augmented by 81% on the yogurt *vs* control diet ($P<0.001$, Figure 2), and this was reflected in a markedly greater reduction in waist circumference (−3.99±0.48 *vs* −0.58±1.04 cm, $P<0.001$, Figure 3). Moreover, the fraction of fat lost from the trunk was higher on the yogurt diet *vs* control ($P<0.005$). Participants on the yogurt diet exhibited a significant increase in circulating glycerol (22.3%,

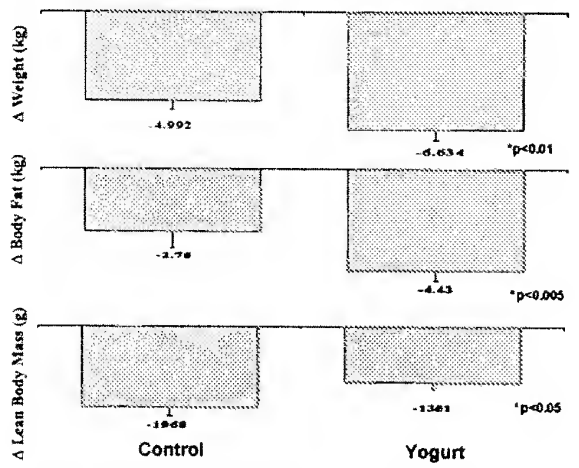

Figure 1 Effects of dietary treatments on body weight composition. Body weight is shown in the top panel, body fat loss (as measured by DEXA) is shown in the middle panel and change in lean body mass (as measured by DEXA) is shown in the bottom panel.

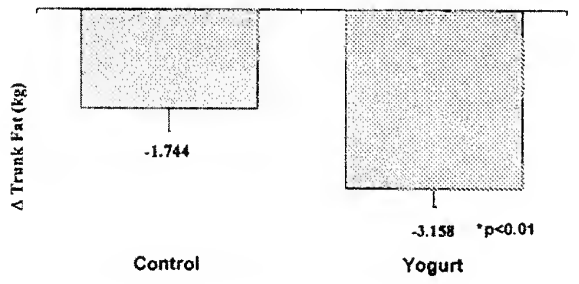

Figure 2 Effects of dietary treatments on trunk fat loss.

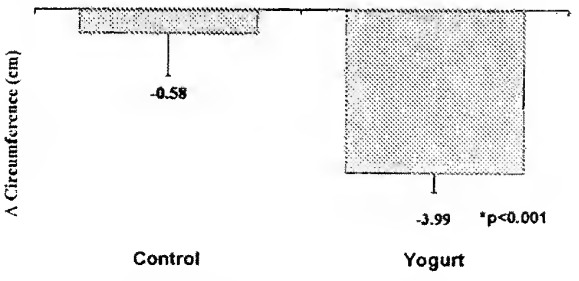

Figure 3 Effects of dietary treatments on waist circumference.

$P<0.001$), indicating an increase in lipolysis in individuals on this diet, while there was no significant change in circulating glycerol in participants on the control diet (Figure 4).

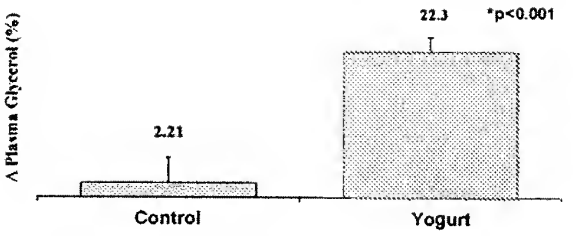

Figure 4 Effects of dietary treatments on circulating glycerol levels. Change in glycerol (%) is calculated as the change from week 0 to week 12 divided by the original (week 0) value and multiplied by 100.

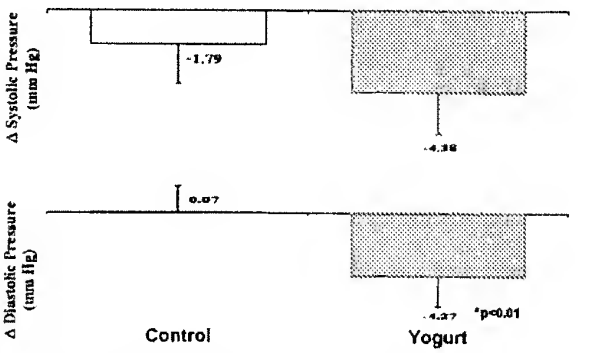

Figure 5 Effects of dietary treatments on systolic (top panel) and diastolic (bottom panel) blood pressure.

There were no significant effects of any of the diets on systolic pressure (Figure 5, top panel) or circulating lipids, while the patients on the yogurt diet exhibited a significant reduction in diastolic pressure ($P<0.01$, Figure 5, bottom panel), consistent with the modest blood pressure lowering effects of both weight loss and dairy ingestion anticipated in overweight, normotensive individuals.[2]

Discussion

Data from this study confirm and extend our previous observations that inclusion of dairy products, in this case three six-ounce servings of yogurt, in the daily diet markedly augments fat loss secondary to modest energy restriction and results in a selective increase in the loss of central adiposity. Indeed, these results are remarkably consistent with our previous clinical trial observations,[11] in which a dairy-rich diet augmented fat loss by 64%, comparable to the 61% augmentation observed in the present study, and increased the fraction of fat lost from the central region of the body from 19 to 66%, quite similar to the increase in loss of central adiposity in the present study however, due to the longer duration (24 *vs* 12 weeks) of the previous trial, total weight and fat loss were ~75% greater in the aforementioned trial.[11] Further, the significant increase in circulating

glycerol, indicative of increased lipolysis, is consistent with our previous observations of the antilipolytic effect of 1,25-dihyroxyvitamin D in human adipocytes and corresponding increases in lipolysis in animals fed high-calcium diets.[1,4,5,9,10,18]

These data add to a growing body of observational and clinical observations that support a role for dietary calcium and dairy foods in controlling excess adiposity. Indeed, a number of recent observational studies have noted an inverse relationship between dietary and/or dairy intake and body fat in both younger and older women,[19–21] African-American women,[21] and children.[22–24]

Evaluating the effects of intact food products, rather than supplements or pharmacological compounds, presents a unique set of problems in clinical trials. Ideally, clinical studies are conducted in a double blind fashion; however, when testing food products, it can be impossible to provide a metabolically inert yet indistinguishable product to use as a placebo. Hence, a control food product was utilized in lieu of a true placebo in this trial. In choosing the control product, a number of factors were considered. These are listed with comments in Table 3. In addition to the sugar-free gelatin snack, which was ultimately chosen, rice milk puddings, granola bars and a pill placebo control were considered. These were rejected on the basis of palatability, sensory characteristics and difficulty of blinding. The gelatin snack was chosen because of its similarity in eating usage (as snack or part of meal), sensory properties (fruit flavors, smooth texture and chilled product) and believability in a diet plan. The greatest concern in using the gelatin snack was that by providing an extremely low-energy density control product, satiety might be induced on fewer calories, resulting in greater weight loss in the control group than would be expected. Although this increased the possibility of a Type I error, this was deemed acceptable.

Melanson *et al*[25] recently reported that calcium intake is positively correlated with whole-body fat oxidation measured in a whole-room calorimeter, with significant effects noted over a 24-h period, during sleep, and during light exercise. Davies *et al*[26] and Heaney *et al*[6,27] have analyzed data from nine studies, including three controlled trials and six observational studies, of calcium intake in which body weight could be assessed as a secondary outcome. Overall, increased calcium intake was consistently associated with reduced indices of adiposity (body weight, body fat and/or weight gain); the aggregate effect was each 300 mg increase in daily calcium intake associated with a 3 kg lower weight in adults and a 1 kg decrease in body fat in children.

Larger population studies similarly support relationship between dietary calcium, dairy and indices of adiposity. Epidemiological observations from NHANES I,[28] NHANES III,[1] The Quebec Family Study,[12] The Continuing Study of Food Intake by Individuals (CSFII[13]), the CARDIA (Coronary Artery Risk Development in Young Adults) study[14] and the HERITAGE Family Study[15] all support an inverse relationship between dietary calcium and/or dairy intake and body fat, BMI or obesity incidence. Moreover, Drapeau *et al*[29] recently reported a significant inverse relationship between changes in body fat and changes in fat-free and low-fat milk consumption in a 6-y follow-up of the Quebec Family Study. However, two recent reviews have pointed out the need for further direct clinical evidence for confirmation of this population-based evidence.[30,31]

Notably, the inverse relationship between dietary calcium and obesity in the CARDIA study was explained solely by dairy intake and was not altered by adjustment for dietary calcium,[14] indicating an additional effect of dairy beyond the mechanisms already cited for dietary calcium in modulating adiposity and obesity risk. This is consistent with our previous observations in rodent studies[1,5,9,10] and clinical trial[11] demonstrating that dairy exerts a greater effect on adiposity than does an equivalent amount of calcium from supplemental or fortified sources. Although the mechanism for this additional effect is not clear, we have previously proposed that it may be attributable, in part, to additional dairy bioactive compounds, such as angiotensin converting enzyme inhibitors, which may act on the adipocyte renin–angiotensin system, as well as to the high concentration of branched-chain amino acids in dairy.[3,4]

Our previous observations of a greater loss of central adiposity on dairy-rich diets in mice[1,5] and clinical trial,[11] which are supported by results of the present study, have been perplexing. However, recent studies demonstrating a potential role for autocrine production of cortisol by adipose tissue in the generation of truncal obesity have prompted us to explore whether the increase in calcitriol, which occurs in response to low-calcium diets, might play a regulatory role in adipocyte cortisol production.

Table 3 Factors influencing choice of control food product

Relevant attribute	*Comments*
Eating occasion/time of eating	Control product eating occasions and frequency should match study product
Sensory characteristics	Similar organoleptic characteristics are desired—sweetness, temperature, texture, etc
Palatability	Should be similar to avoid compliance differences
Caloric content, macronutrients, energy density	Should match study product as closely as possible
Other (GI, insulin response, digestive system disturbances)	Differences in physiological responses that may affect weight loss should be minimized
No active ingredients	Avoid adding variables
Blind to subjects and investigators	Prevent bias

Human adipose tissue expresses significant 11-β-hydroxysteroid dehydrogenase-1 (11-β-HSD-1), which can generate active cortisol from cortisone, and visceral adipose tissue exhibits greater 11-β-HSD-1 expression than does subcutaneous adipose tissue.[32,33] Further, selective overexpression of 11-β-HSD-1 in white adipose tissue of mice results in central obesity,[34,35] while homozygous 11-β-HSD-1 knockout mice exhibit protection from features of the metabolic syndrome.[36] Preliminary data from this lab data demonstrate that calcium agonists, including calcitriol, exert rapid and substantial (3- to 6-fold) stimulation of cortisol production by human adipocytes,[37,38] suggesting that the observed selective loss of central adiposity on high-calcium and high-dairy diets may be attributable, in part, to a reduction in cortisol production by visceral adipocytes. However, these preliminary data are at present only suggestive, as they are derived solely from *in vitro* studies. Nonetheless, they do provide a plausible mechanism for a heretofore inexplicable phenomenon.

In summary, isocaloric substitution of yogurt into the diet of obese adults results in a repartitioning of energy and body composition, resulting in reductions in body weight, total adiposity and central adiposity and an improved metabolic profile during energy restriction. *In vitro* data obtained in human adipocytes suggest that the partially selective effect on central adiposity may result, in part, from reductions in adipocyte cortisol production secondary to suppression of calcitriol levels on the higher calcium diets.

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